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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/258,132
Filing Date: February 26, 1999
Appellant(s): GOELET ET AL.

J. David Ellet, Jr.
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed June 26, 2008 appealing from the Office action mailed May 22, 2008.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The following are the related appeals, interferences, and judicial proceedings known to the examiner which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal:

Interference No. 103,562

Interference No. 103,739

U.S. Patent Nos. 5,888,819, 6,004,744, and 6,537,748 are the subject of a pending patent infringement lawsuit: Beckman Coulter Inc. and Orchid Cellmark Inc. v. Sequenom, Inc., No. 08 CV 1013 W POR (S.D. Cal. 5 June 2008).

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

5,332,666	PROBER et al	07-1994
4,656,127	MUNDY et al	04-1984
4,962,020	TABOR et al	10-1990
EP 0412883A1	COHEN et al	13 February 1991
FR 2,650,840	COHEN et al	15 February 1991
WO 90/11372	DAVIS et al	04 October 1990

SOUTHERN, E. M., Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis, Journal of Molecular Biology, 1975, 98: 503-517.

CONNER, B. J. et al, Detection of sickle cell [3S-globin allele by hybridization with synthetic oligonucleotides, Proceedings of the National Academy of Sciences USA, January 1983. 80: 278-282.

STRYER, L. Biochemistry, third edition, 1988, page 169, W.H. Freeman and Company, New York.

Zeta-Probe® Blotting Membranes, Instruction Manual, (LIT234 Rev C), undated, 29 pages, Bio-Rad Laboratories, Hercules, California.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

A. Claims 64, 66, 67, 69, and 70 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Cohen et al (EP 0412883) or Cohen et al (FR 2,650,840), each in view of Davis (WO 90/11372).

It is noted that EP 0412883A1 claims priority to application 8910802, which issued as and is identical in content to FR 2,650,840. An English translation of FR 2,650,840 was filed in the IDS of June 8, 1999.

Cohen teaches a method for determining the identity of one or more nucleotide bases in a target nucleic acid wherein the method comprises contacting a single-stranded target nucleic acid with an oligonucleotide primer to form a duplex between the complementary target nucleic acids and the oligonucleotide primer, wherein the oligonucleotide primer hybridizes immediately 3' of the nucleotide base to be determined in the target nucleic acid; contacting the resulting duplexes with a solution containing four different terminators, each terminator labeled with a different detectable moiety; extending the primer with the terminator, and determining the identity of the incorporated terminator to thereby determine the identity of the nucleotide base (see pages 4 and 5). Cohen (page 6) states that "if the four blocking bases are marked by means of different markers, the four blocking nucleotides are advantageously detected at the same time." In the method of Cohen, only terminator nucleotides are present in the extension reaction – the reaction does not contain dATP, dCTP, dGTP or dTTP (see, for instance, Example 1). Cohen does not teach performing the primer extension reaction using multiple primers, each comprising a different affinity moiety, and affinity

separating the extended primers by contacting the extended primers with an affinity group attached to a solid support.

However, Davis teaches a method for determining the identity of one or more nucleotide bases in a target nucleic acid molecule wherein the method comprises contacting a single stranded target nucleic acid molecule with an oligonucleotide primer to form a duplex between the oligonucleotide primer and complementary target nucleic acids; contacting the duplexes with a solution containing labeled dNTPs, labeled with a different detectable moiety; extending the primer with the dNTPs such that if the primer is perfectly complementary with the target nucleic acid, an extension product is formed, but if the primer contains a mismatch at or near the 3' end of the primer, an extension product is not formed, and detecting the presence of an extension product in order to determine the identity of a nucleotide base (see pages 3-4). Davis teaches that the identity of multiple nucleotides can be determined simultaneously by using a mixture of different oligonucleotides, each oligonucleotide comprising a unique tail (i.e., an "affinity moiety"). Following the extension reaction, the primer extension/target nucleic acid duplexes are denatured, and the primer extension products are hybridized to a solid support having bound thereto nucleic acid capture probes complementary to the primer tail (i.e., an "affinity group attached to a solid support"). The unique tail allows for the primers to be immobilized at specific locations on the support (see pages 4-5). Davis teaches that the use of multiple primers, with different tail sequences allows for the simultaneous analysis of multiple sequences and improves the speed and sensitivity of the detection method (see page 21).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Cohen so as to have used multiple primers, each having a different tail (i.e., each comprising a different affinity moiety) and to have separated the primer extension products from the reaction medium by contacting the extension products with a solid support having immobilized thereon a capture probe complementary to the tail sequence (i.e., an affinity group complementary to the affinity moiety of the primer) in order to have accomplished the objectives set forth by Davis of allowing for the analysis of multiple sequences simultaneously and of providing a more rapid and sensitive means for determining the identity of a nucleotide.

With respect to claim 66, Cohen teaches that the terminator (or "blocking nucleotide") is a dideoxynucleotide (see page 5).

With respect to claim 67, Cohen teaches that the terminator comprises one or more of ddATP, ddCTP, ddGTP or ddTTP (see pages 7 and 8).

With respect to claims 69 and 70, Cohen teaches that the terminator may be labeled with a fluorophore, or chromophore, isotope, enzyme or antibody (see page 5).

B. Claim 68 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Cohen et al (EP 0412883) or Cohen et al (FR 2,650,840), each in view of Davis (WO 90/11372) and Prober (U.S. Patent NO. 5,332,666).

The teachings of Cohen and Davis are presented above. The combined references do not teach performing the primer extension reaction using a terminator that comprises arabinoside triphosphate.

However, Prober teaches methods for determining a nucleotide sequence wherein the method comprises performing a primer extension reaction using a terminator. Prober teaches that the terminator may contain an arabinose as the sugar group and provides a number of examples of terminators comprising an arabinoside triphosphate (see column 18).

In view of the teachings of Prober, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the primer extension step of Cohen so as to have used a terminator comprising an arabinoside triphosphate because this would have provided an equally effective terminator for the primer extension reaction and an equally effective means for determining the identity of a nucleotide base in a target nucleic acid.

C. Claim 71 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Cohen et al (EP 0412883) or Cohen et al (FR 2,650,840), each in view of Davis (WO 90/11372) and Tabor (U.S. Patent No. 4,962,020).

The teachings of Cohen and Davis are presented above. The combined references do not teach including pyrophosphatase in the primer extension medium.

However, Tabor (columns 15-16) teaches including pyrophosphatase in primer extension reactions. The reference teaches that pyrophosphatase removes pyrophosphate which builds up during extension reactions. Specifically, Tabor (column 14) teaches that in the presence of pyrophosphate, DNA polymerase will add pyrophosphate to the 3' terminal nucleotide, causing the release of dideoxynucleoside

5'-triphosphates. As stated by Tabor (column 15, lines 1-2), "(t)his reaction has the effect of removing the block at the 3' terminus."

In view of the teachings of Tabor, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have further modified the method of Cohen so as to have included pyrophosphatase in the primer extension reaction medium in order to have achieved the expected benefit of eliminating pyrophosphorolysis activity of DNA polymerase and thereby reducing the probability that the labeled terminator would be removed and that unlabeled dideoxynucleotides would be released into the reaction medium. Thereby, the ordinary artisan would have been motivated to have include pyrophosphatase in the extension reaction in order to have ensured the accuracy and sensitivity of the method for determining the identity of a nucleotide.

(10) Response to Argument

A. Rejection of claims 64, 66, 67, 69 and 70 under 35 USC § 103 as unpatentable over Cohen ('840 or '883) in view of Davis.

Prior to addressing Appellants arguments, it is noted that throughout the brief, Appellants provide their opinion of what one of ordinary skill in the art would or would not have done given the teachings of Cohen and Davis. However, Appellants fail to provide any declaratory evidence to support such allegations. Further, Appellants provide no evidence or other showing to establish that one of ordinary skill in the art would not have had a reasonable expectation of success of modifying the method of Cohen so as to have separated the primer extension products from the reaction medium

by contacting the primer extension products with a solid support having immobilized thereon a capture probe complementary to the primer tail sequence, as is taught by Davis. Given the specific teachings of Davis that separation of primer extension products from the reaction medium by contacting the primer extension products with a solid support having immobilized thereon a capture probe complementary to the tail sequence provides the advantages of allowing for the analysis of multiple sequences simultaneously and of providing a more rapid and sensitive means for determining the identity of a nucleotide, and given the extensive guidance provided by Davis for accomplishing such a method, one of ordinary skill in the art would have both been motivated to have modified the method of Cohen and would have had more than a reasonable expectation of success of modifying the method of Cohen so as to have included such a separation step.

In the brief, Appellants traversed the rejection by stating that Cohen directly teaches away from the hypothetical combination of the method of Cohen and the method of Davis. Appellants state that Cohen teaches that the advantage of the process described therein was that the process did not require immobilization of the nucleic acid on a membrane. Appellants point to page 2, lines 10-17 and page 4, lines 14-17 of Cohen as providing support for this argument. Appellants conclude that the teachings of Cohen would have directly led one of ordinary skill in the art away from “any technique which shared the requirement of immobilization of nucleic acid on a membrane."

Appellants' arguments have been fully considered but are not persuasive. At page 2, lines 10-17, Cohen addresses the effect of temperature on the specificity of

hybridization using probes to "detect a mutation involving a single base." These teachings are not considered to be relevant to the method of Davis because the method of Davis does not require the use of a probe to directly hybridize to and detect a single base mutation in a target nucleic acid. Rather, the method of Davis involves only performing the step of hybridizing primer tails to immobilized capture probes that are fully complementary to the primer tails (i.e., hybridization is not with a target nucleic acid comprising a single base mutation). At page 4, lines 14-17, Cohen discusses prior art techniques which require immobilization of a target nucleic acid on a membrane and "marking" of a probe. The cited teachings of Cohen do not teach away from the method of Davis because the method of Davis does not require directly immobilizing the target nucleic acid on a membrane and does not require contacting the immobilized target nucleic acid with a "marked" (i.e., labeled) probe t thereby detect a mutation. Rather, the method of Davis requires contacting the target nucleic acid with a primer having a tail, extending the primer to form a primer extension product, and contacting the tail of the primer extension product with a capture probe immobilized onto a solid support.

At page 14 of the brief, Appellants cite Cohen page 3, lines 10-17 in support of their conclusion that Cohen teaches away from any method that requires immobilization of nucleic acids. However, the statements of Cohen at page 3, lines 10-17 must be taken in context with the preceding teachings of Cohen at page 3, lines 4-9. Therein, Cohen states:

> "By selecting suitable hybridization and rinsing conditions (specific for each system), hybridization by means of marked oligonucleotides can be achieved

only in case of perfect equivalence (the difference of a single nucleotide, particularly at the site of the mutation, results in destabilization of the hybridization). However, these various techniques all have a certain number of disadvantages: - the temperature conditions are difficult to master to achieve suitable hybridization; - the mandatory presence of a restriction site may be required; - the nucleic acid is immobilized on a membrane (Southern blot) " (emphasis added).

Accordingly, it is acknowledged that Cohen teaches the difficulties associated with using hybridization techniques to directly detect a single nucleotide mutation. Cohen may be viewed as teaching away from using this methodology in which hybridization of a probe to a target nucleic acid is performed to directly detect a single nucleotide variation in the target nucleic acid because the conditions of hybridization are critical to the accuracy of the detection step. Immobilization of the target nucleic acid to a solid support is known to interfere to some degree with the kinetics of the hybridization process and thereby with the specificity of hybridization. Again, the criticality of the hybridization process is important here because it is the hybridization step alone which is relied upon solely to distinguish between nucleic acids having the single nucleotide mutation from nucleic acids that do not have the single nucleotide mutation. This is in contrast to the method of Davis wherein the hybridization step does not serve to distinguish between fully complementary nucleic acids and nucleic acids that include a single point mutation.

Thus, Appellants arguments do not fairly characterize the teachings of Cohen. There are no teachings in Cohen which make a general conclusion that “any technique which shared the requirement of immobilization of nucleic acid on a membrane" should be avoided. Cohen teaches only the disadvantages of using immobilized nucleic acids to directly detect a point mutation by hybridizing a probe to the nucleic acid wherein it is the hybridization step itself that detects the point mutation. All of the teachings of Cohen regarding the disadvantages of immobilized nucleic acids are limited to methods in which hybridization with a marked (labeled) probe serves to directly detect the presence of a target nucleic acid comprising a point mutation. Cohen does not teach away from all techniques of immobilizing nucleic acids, such as immobilizing nucleic acids for the alternative purpose of capturing a primer extension product.

Appellants assert that the method of Davis is such a technique that involves immobilization of nucleic acids on a membrane, “from which the Cohen et al patent taught away.” Appellants argue that Davis teaches attaching specific oligonucleotides to a substrate. It is stated that Cohen drew no distinction between reversibly immobilizing nucleic acid on a membrane and irreversibly immobilizing nucleic acid on a membrane. Appellants also state that the method of Davis involves detecting a hybrid complex immobilized on a membrane in which a first component of the hybrid complex is irreversibly bound to a membrane. It is asserted that one of ordinary skill in the art would have appreciated that the method of Davis required a feature that Cohen characterized specifically as a disadvantage.

These arguments have also been fully considered but are not persuasive. There are no statements in Cohen which support a conclusion that the methodology disclosed by Davis would be considered a "disadvantage." Again, the teachings of Cohen regarding immobilization of nucleic acids are made in the context of methods in which hybridization between an immobilized target nucleic acid and a labeled nucleic acid probe is performed under conditions in which the hybridization step itself distinguishes between nucleic acids that contain a single nucleotide mutation and nucleic acids that do not contain a single nucleotide mutation. The teachings of Cohen do not address the use of hybridization following primer extension. In particular, Cohen does not teach away from the method of Davis in which following primer hybridization and primer extension in solution, the primer extension product is immobilized in order to facilitate the separation of the primer extension product from the reaction components. As set forth by Davis, this methodology provides the advantage of allowing for the analysis of multiple sequences simultaneously and of providing a more rapid and sensitive means for determining the identity of a nucleotide. Thus, Davis specifically provides the motivation for combining the teachings set forth therein with the teachings of Cohen. As stated in MPEP 2145:

"A prior art reference that "teaches away" from the claimed invention is a significant factor to be considered in determining obviousness; however, "the nature of the teaching is highly relevant and must be weighed in substance. A known or obvious composition does not become patentable simply because it has been described as somewhat inferior to some other product for the same use." In re Gurley, 27 F.3d 551, 554, 31 USPQ2d 1130, 1132 (Fed. Cir. 1994) (Claims were directed to an epoxy resin based printed circuit material. A prior art reference disclosed a polyester-imide resin based printed circuit material, and taught that although epoxy resin based materials have acceptable stability and some degree of flexibility, they are inferior to polyester-imide resin based materials. The court held the claims would have been obvious over the prior art because the reference taught epoxy resin based material was useful for Appellant's purpose, Appellant

did not distinguish the claimed epoxy from the prior art epoxy, and Appellant asserted no discovery beyond what was known to the art.)."

In the present situation, when one weighs the nature of the teachings of Cohen, it is readily apparent that Cohen does not in fact teach away from the claimed invention. Cohen teaches only that methods which directly detect a point mutation by using an immobilized nucleic acid and a labeled probe are inferior to methods which detect a point mutation using a primer extension assay. Cohen does not teach that primer extension assays cannot or should not be combined with an additional step in which primer extension products are subsequently immobilized to facilitate their separation and detection.

Cohen (page 2, lines 19-35) describes prior art methods using long-probes and short-probes. The brief provides a review a such methods. Appellants describe a "long-probe" technique of Southern in which a restriction enzyme is used to cleave a DNA, the cleaved DNA is size separated by electrophoresis, the size-separated DNA is immobilized onto a membrane, and hybridized to a "long-probe." Appellants state that one would have understood that the long-probe technique described in Cohen et al referred to the Southern blot technique in which a nucleic acid irreversibly bound to a membrane is hybridized to a radioactively labeled oligonucleotide probe. Appellants further cite Stryer as teaching a method of detecting a single nucleotide polymorphism by digesting a DNA with a restriction enzyme, separating the DNA by gel electrophoresis, performing Southern blotting, and detecting size separated fragments immobilized on a membrane using a labeled probe. Appellants conclude that the long-

probe technique described by Cohen refers to the method disclosed on page 169 of the Stryer textbook. Appellants state that Cohen also discloses prior art methods of detecting mutations using short-probes. It is asserted that one would understand the short probe technique referred to by Cohen encompasses the allele-specific hybridization methods disclosed in the Conner et al reference. Appellants cite Cohen at page 2, line 19 through page 3, line 17 as teaching the disadvantages of the long-probe and short-probe techniques:

> "Thus, to detect a mutation involving a single base, depending on the case generally two types of probes can be used: nucleic acid probes called long probes, generally over 150 nucleotides, or nucleic acid probes called short probes, generally between 17 and 24 nucleotides. If the mutation occurs at a site recognized specifically by an enzyme called a restriction enzyme, the Southern blot technique can be used. This technique includes stages of isolating the DNA, digestion by the restriction enzyme, electrophoresis on gel, transfer onto a membrane, and hybridization by means of a long probe involving the region of the mutation; after washing and autoradiography, analysis of the size of the fragments obtained permits confirmation or invalidation of the presence of the mutation. This very cumbersome process requires that the mutation involve a restriction site. If this is not the case, a short nucleotide probe of 17 to 24 nucleotides can be synthesized, the center of which coincides with the mutation that one wishes to detect. By selecting suitable hybridization and rinsing conditions (specific for each system), hybridization by means of marked oligonucleotides can be achieved only in case of perfect equivalence (the difference of a single nucleotide, particularly at the site of the mutation, results in destabilization of the hybridization).
>
> However, these various methods all have a certain number of disadvantages:

- the temperature conditions are difficult to master to achieve suitable hybridization;
- the mandatory presence of a restriction site may be required;
- the nucleic acid is immobilized on a membrane (Southern blot)" (emphasis added).

These arguments have also been fully considered but are not persuasive. It is agreed that Cohen teaches the advantages of their method over prior art techniques of using hybridization of a long-probe or short-probe to a target nucleic acid to detect the presence of a mutation in a target nucleic acid. However, Cohen does not teach that that the method disclosed therein cannot be combined with methods of hybridization performed for alternative purposes, such as for the capture of a primer extension product.

Appellants state that the long-probe and short-probe technique have the disadvantages that the "temperature conditions are difficult to master to achieve suitable hybridization." However, this statement is taken out of context. The statement of Cohen regarding temperature conditions is made with respect to methods "to detect a mutation involving a single base" (page 2 of Cohen). Thus, Cohen states that the temperature conditions are difficult to master when one is trying to employ the short-probe technique to detect a specific target nucleic acid having a point mutation and to distinguish this target nucleic acid from other nucleic acids that do not have the point mutation. Cohen does not teach that all methods of hybridization are difficult to perform because of the requirement to select suitable hybridization conditions.

In contrast to the teachings of Cohen regarding the use of long-probes and short-probes, in the method of Davis, hybridization occurs between an affinity tail of the primer and a fully complementary immobilized capture nucleic acid – i.e., not between nucleic acids that must be distinguished from one another based on the presence of a single point mutation present in the center of the short-probe. Given the guidance provided by Davis, it would have been well within the skill of the art to have selected suitable conditions to hybridize the tail of the primer to a complementary immobilized capture probe. Further, Cohen teaches that the long-probes used in the method of Southern are generally over 150 nucleotides. However, in the method of Davis, the tail portion is 14 nucleotides in length and the complementary oligonucleotide is a polymer that consists of repeating units of 14 nucleotides. As stated by Davis (page 22), "The use of such repeating units of complementation favorably affects the kinetics of hybridization, further increasing the speed and the sensitivity of the assay." Thereby, it is maintained that it would have been well within the skill of the art to have selected suitable hybridization conditions for hybridizing and immobilizing the primer tail, since Davis specifically provides the guidance for performing such methods. Additionally, Davis (page 21) teaches numerous advantages of including this additional step of immobilizing the tails of the primer extension products, stating, for example, that "(i)mprovements to the speed and sensitivity of the assay for the extension product are achieved using such primers having tails." Davis (page 21) also states that because the complementary oligonucleotides may be synthesized inexpensively in great quantity and can therefore be applied to the substrate in great excess, the rate and amount of

hybridization between the tail region of the extension product and the complementary immobilized oligonucleotide is increased.

Further, it is noted that Appellants have relied on the teachings of Stryer to support their allegation of the disadvantages associated with using restriction enzyme digestion followed by electrophoresis and hybridization to detect a mutation. However, Stryer in fact teaches that this is an effective technique for detecting a point mutation. Stryer does not point to any insurmountable problems observed when performing hybridization of the "long-probe" to the restriction enzyme digested DNA. Rather, Stryer teaches the successful application of this technique to detect a point mutation associated with single-cell anemia.

Appellants state that Cohen specifically teaches the disadvantage of immobilizing a nucleic acid on a membrane in a Southern blotting technique. Appellants state that the method of Davis requires irreversibly binding tail-complementary oligonucleotides to a membrane and applying extension products to the membrane. Appellants conclude that Cohen teaches away from this methodology. This argument is not persuasive because the teachings of Cohen relate to the immobilization of the target nucleic acid irreversibly to the membrane in the method of Southern blotting. Cohen does not teach away from immobilizing a capture probe that is complementary to a tail region of the primer extension product, as is taught by Davis.

At page 24 of the brief, Appellants state that the long-probe technique does not require hybridization specificity to detect a point mutation and again cite Cohen as teaching that "the temperature conditions are difficult to master to achieve suitable

hybridization." Appellants assert that this teaching of Cohen also applies to methods using long-probes. However, the teachings of Cohen are not consistent with such an interpretation. While in the concluding sentence "the temperature conditions are difficult to master to achieve suitable hybridization," Cohen does not specify that this refers to hybridization with a long-probe. The preceding paragraph must be considered since it provides the details regarding this conclusory statement. In the preceding paragraph, Cohen states that "(i)f this is not the case, a short nucleotide probe of 17 to 24 nucleotides can be synthesized, the center of which coincides with the mutation that one wishes to detect. By selecting suitable hybridization and rinsing conditions (specific for each system), hybridization by means of marked oligonucleotides can be achieved only in case of perfect equivalence (the difference of a single nucleotide, particularly at the site of the mutation, results in destabilization of the hybridization)." Thus, the statement "the temperature conditions are difficult to master to achieve suitable hybridization" briefly summarizes the statement above regarding hybridization with short-probes. One of ordinary skill in the art reading this complete disclosure would recognize that Cohen taught that the temperature conditions are difficult to master to achieve suitable hybridization with a short-probe in which the center nucleotide included a mutation that one wished to detect. Thus, this disclosure in Cohen does not teach away from the method of Davis in which a capture probe fully complementary to a tail region of a primer is used to hybridize to and thereby immobilize a primer extension product.

Appellants assert that Cohen teaches away from immobilization of nucleic acids on a membrane and thus teaches away from the hypothetical combination of the process of Cohen with the method of Davis, since the method of Davis involves immobilizing a nucleic acid on a membrane. This argument has been fully considered but is also not persuasive. At page 3, Cohen teaches the disadvantage of "the nucleic acid is immobilized on a membrane (Southern blot)." One of ordinary skill in the art would readily appreciate the differences between the immobilization technique required for Southern blotting as compared to the immobilization technique required by the method of Davis. As stated by Cohen (page 2 through page 3), Southern blotting is a very "cumbersome process," requiring the isolation of DNA, the digestion of DNA by a restriction enzyme, electrophoresis of DNA on a gel, transfer of the target DNA onto a membrane, hybridization of the DNA with a long-probe, washing and autoradiography. This is in contrast to the method of Davis which does not require isolation of target DNA, digestion of target DNA with a restriction enzyme, electrophoresis of target DNA in a gel, or transfer of the target DNA from the gel to a membrane. The method of Davis requires only hybridization of the tail region of a primer extension product to an immobilized probe comprising a sequence complementary to the tail region. Thus, the teachings of Cohen regarding the disadvantages of immobilization of nucleic acids by Southern blotting do not apply to the method of Davis.

Appellants cite Mundy as teaching an alternative method for detecting a mutation. It is stated that "a person of ordinary skill in the art would have understood the method of the Mundy '127 patent in embodiments characterized in the patent as

preferred, like the long-probe technique and the short-probe technique in spite of differences noted in the Mundy patent itself, to have involved detecting a hybrid nucleic-acid complex immobilized on a membrane, in which a first component of the hybrid complex would have been a nucleic acid effectively irreversibly bound to the membrane by baking or a process of similar effect and a second component of the hybrid complex would have been a labeled oligonucleotide reversibly bound to the first component by hybridization."

This argument has also been fully considered but is not persuasive. Again, the method of Mundy is distinct from the method of Davis. There are no teachings in Cohen or Mundy which teach away from a method of separating primer extension products from other reactants by hybridizing the primer extension product to an immobilized capture probe, as is taught by Davis.

Appellants state that Davis teaches that the oligonucleotide probes are bound to discrete locations on a nylon membrane identified by the brand name "ZetaProbe." It is stated that the oligonucleotide probes are preferably firmly bound to the substrate. Appellants again cite Cohen (page 6, lines 29-33) as teaching that an advantage of the method disclosed therein is that the method did not require immobilization of nucleic acid on a membrane. It is argued that Cohen did not draw a distinction between reversibly and irreversibly immobilizing nucleic acids on a membrane. Appellants conclude that persons of ordinary skill in the art would thus have deemed it in no way obvious to combine the process of Cohen with the method of Davis.

These arguments have also been fully considered but are not persuasive. While Appellants offer their opinion that one of ordinary skill in the art would not have combined the process of Cohen with the method of Davis, Appellants provide no evidence to substantiate their opinion of what the ordinary artisan would have done. Regarding the teachings of Cohen at page 6, lines 29-33, Cohen states: "One specific advantage of the process pursuant to the invention is that it defines the operative conditions independently of the nucleotide base to be identified, and does not require immobilization of the nucleic acid." Accordingly, taken in context, Cohen teaches the advantage of the method disclosed therein is that with respect to the step of detecting a mutation, this step does not require the immobilization of the nucleic acid. This property of the method of Cohen is also a property of the method of Davis. In the method of Davis, the primer extension step which detects the mutation occurs independently of a step of immobilizing the target nucleic acid. In the method of Davis, the target nucleic acid potentially comprising the mutation is not itself directly immobilized onto a membrane. Thereby, Cohen is not considered to teach away from the method of Davis.

Moreover, it is noted that while Cohen teaches advantages of the method disclosed therein, such teachings do not constitute a teaching away from the combination of Cohen and Davis. There are no teachings in Cohen which indicate that the method disclosed therein cannot be modified or combined with alternative methods, such as the method of Davis. Cohen et al teaches only why their methodology is advantageous over the prior art. However, when considered collectively, the ordinary artisan would have recognized that the combination of Cohen and Davis would have

provided numerous advantages over the prior art, including the advantages of allowing for the analysis of multiple sequences simultaneously and of providing a more rapid and sensitive means for determining the identity of a nucleotide.

Appellants state that the method of Davis would have shared the disadvantage of temperature conditions being hard to achieve for suitable hybridization. This argument is not convincing because the temperature conditions that Cohen notes are hard to achieve pertain to those conditions that allow one to perform a hybridization step in which a detection probe hybridizes to a fully complementary target nucleic acid and does not hybridize to a target nucleic acid that includes a point mutation. The method of Davis does not require such a step. Rather, in the method of Davis, the immobilized capture probe is fully complementary to the primer tail. There is no requirement in the method of Davis to distinguish between hybridization of the probe to fully complementary nucleic acids and nucleic acids that have a single point mutation. Moreover, Davis clearly teaches that the selection of the appropriate hybridization conditions, including temperature, is well within the skill of the skill of the art and exemplifies methods in which such a hybridization step is readily achieved (see page 47, 52-53, and 58). Davis also teaches the advantages of and the high level of effectiveness obtained with hybridizing primer tails to immobilized capture probes following the primer extension reaction. Again, Davis states that:

> "(i)mprovements to the speed and sensitivity of the assay for the extension product are achieved using such primers having tails. The extension products 27 may be detected by using substrates such as filter paper, nylon, or nitrocellulose 30 spotted with a great excess of oligonucleotide complementary to the tail portion 26. Because such complementary oligonucleotide DNA 32 may be synthesized inexpensively in great quantity and therefore may be applied to the

> substrate in great excess (FIG. 4), the rate and amount of hybridization between the tail portion 26 of the extension product 27 and the complementary oligonucleotide 32 on the substrate is increased as compared to a system employing DNA complementary to the extension product, which DNA generally is nature DNA, is not generally available in great quantity and is usually mixed with other DNA, thereby diluting it."

Appellants state that “a person of ordinary skill in the art would not have attempted to combine the single-base-identification process of the Cohen et al ‘840 French patent with the multiple-allele/multiple-loci method of Davis.” This argument is not persuasive because Appellants fail to provide any declaratory evidence to substantiate this allegation of what or what not a person of ordinary skill in the art would have attempted. It is noted that the legal standard for "reasonable expectation of success" is provided by case law and is summarized in MPEP 2144.08, which notes "obviousness does not require absolute predictability, only a reasonable expectation of success; i.e., a reasonable expectation of obtaining similar properties. See, e.g., In re O'Farrell, 853 F.2d 894, 903, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988)." In the present situation, the guidance and examples provided by Davis provide the ordinary artisan with more than a reasonable expectation of success of effectively achieving the step of hybridizing the tail of the primer to the immobilized capture probes.

Additionally, Appellants arguments are not convincing in view of the fact that Davis and Cohen are analogous art since both the method of Davis and the method of Cohen rely on performing a primer extension reaction to detect a single nucleotide variation. On the other hand, Southern, Stryer, Mundy and Conner do not rely on similar techniques to accomplish the detection of a single nucleotide variation, since Southern,

Stryer, Mundy and Conner each teach detecting a single nucleotide variation in a target nucleic acid by contacting the target nucleic acid with a labeled hybridization probe.

For the reasons stated above, it is maintained that Cohen and Davis when considered as a whole would have lead the ordinary artisan to the claimed invention. Davis provides both the motivation for combining teachings and provides extensive guidance for achieving such a combination. Accordingly, it is maintained that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Cohen so as to have used multiple primers, each having an affinity moiety and to have separated primer extension products from the reaction medium by contacting the extension products with a solid support having capture probes immobilized thereon in order to have accomplished the objectives set forth by Davis of allowing for the analysis of multiple nucleic acid sequences simultaneously and of providing a more rapid and sensitive means for determining the identity of a nucleotide in a nucleic acid sequence.

B. Rejection of claim 68 under 35 USC § 103 as unpatentable over Cohen ('840 or '883) in view of Davis and Prober.

Appellants traversed this rejection by stating that the Prober patent does not overcome the teachings of Cohen et al against the hypothetical combination of Cohen and Davis. It is stated that the arguments regarding the combination of Cohen and Davis apply equally to the present grounds of rejection.

Appellants' arguments regarding the combination of Cohen and Davis have been fully addressed above. Accordingly, the response to Appellants' arguments, as set forth above, apply equally to the present grounds of rejection.

C. Rejection of claim 71 under 35 USC § 103 as unpatentable over Cohen ('840 or '883) in view of Davis and Tabor.

Appellants traversed this rejection by stating that Tabor does not overcome the teachings of Cohen et al against the hypothetical combination of Cohen and Davis. It is stated that the arguments regarding the combination of Cohen and Davis apply equally to the present grounds of rejection.

Appellants' arguments regarding the combination of Cohen and Davis have been fully addressed above. Accordingly, the response to Appellants' arguments, as set forth above, apply equally to the present grounds of rejection.

(11) Related Proceeding(s) Appendix

Copies of the court or Board decision(s) identified in the Related Appeals and Interferences section of this examiner's answer are provided herein.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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